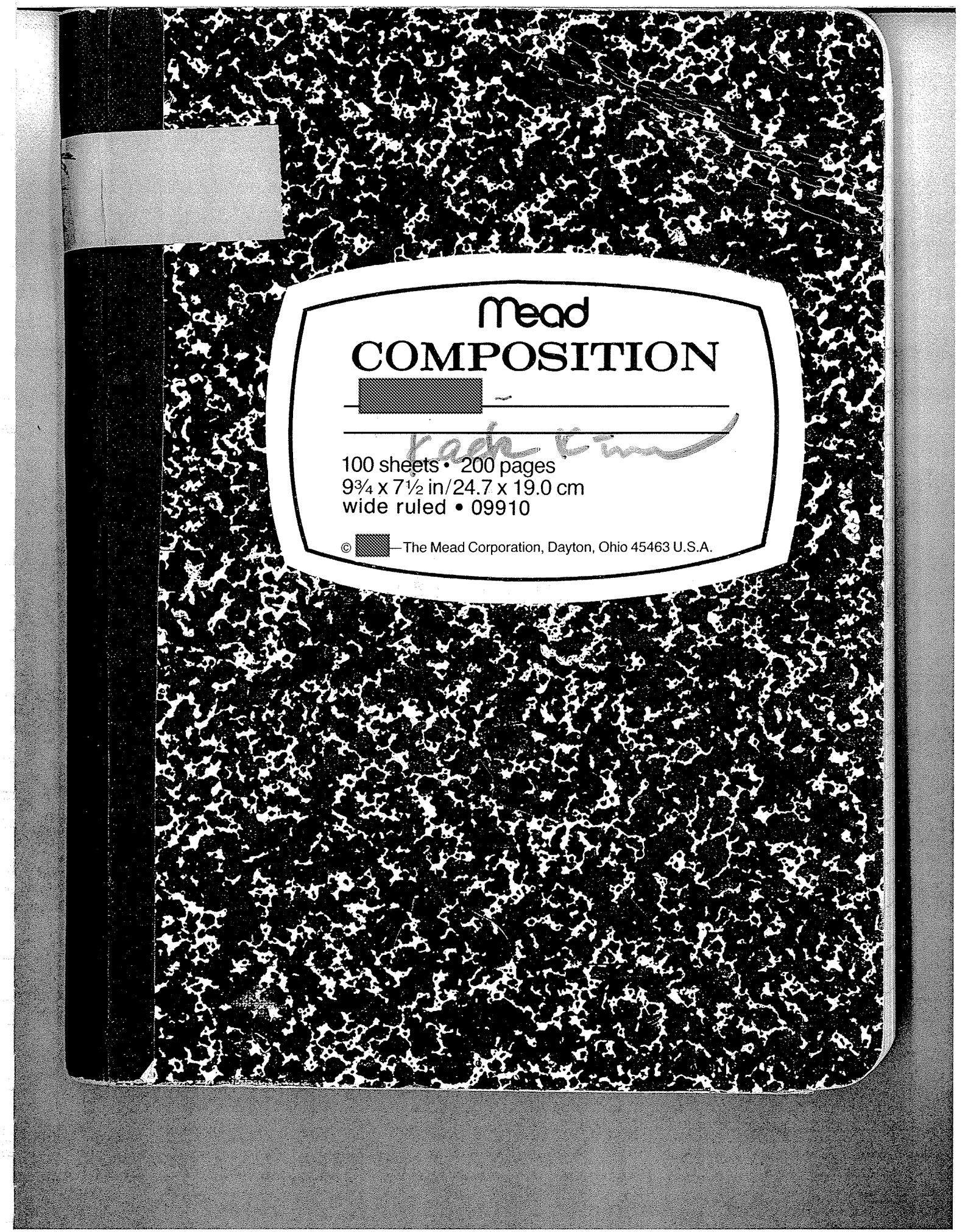
EXHIBIT 18


Mead
COMPOSITION
100 sheets • 200 pages
9¾ x 7½ in/24.7 x 19.0 cm
wide ruled • 09910
© —The Mead Corporation, Dayton, Ohio 45463 U.S.A.

pmel 17 → ← 1st PCR
→ ← 2nd PCR

chicken gene homologous to pmel 17 : Japan

PCR ⊂ human melanocyte RNA → 700, ~~600~~ 500, 400, 300 bp ~~100 bp~~

(2) | pmel17 ⊂ deletion [same donor site, differ acceptor "]

| pMEL17

become smaller (~~600~~ 500 bp) when cloned

run gel [700]

(*) get 700 !! → 900 bp

look p 26

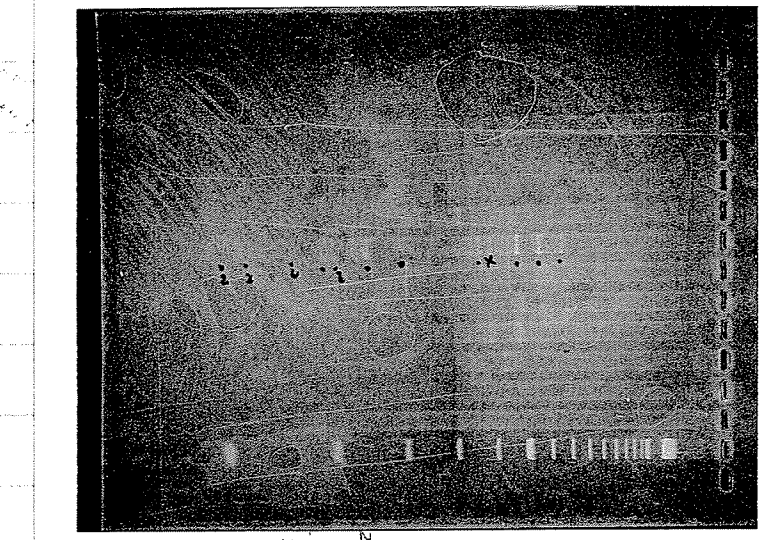
4-1BB

(*) get Jurkat 500 → (filter already made high stringent
Southern [human, Gibbon, mouse DNA]
Genomic DNA cut ⊂ RI

500	cloned partially seq.
380	380 → cloned but (?)
300	300 Ribosomal bindy protein
	200 → (?)
Jurkat	Gibbon

pHA-stimulated human PBL T cell

① MLA poly A+ (Gibbon T cell)
② Jurkat (human T)
③ Molt4 (human T)



F8
Nzg. control
MLA poly-Ac H2
" " H3R
" " 2+3G
" Total H2
" " H3R
" " 2+3G
More 4 Total H2
" " H3R
" " 2+3G
100 bp ladder

KWON000131

10-13h

MLA poly A+ 1+2
" " 1+3R
" " 2+3Tr

" Total RNA 1+2
" " 1+3R
" " 2+3Tr

Mo1+4 "
" "
" "

R8 ~~Poly A+~~ Total RNA 1+2

Negative control

10 μl each, 100 ~ 400 bp

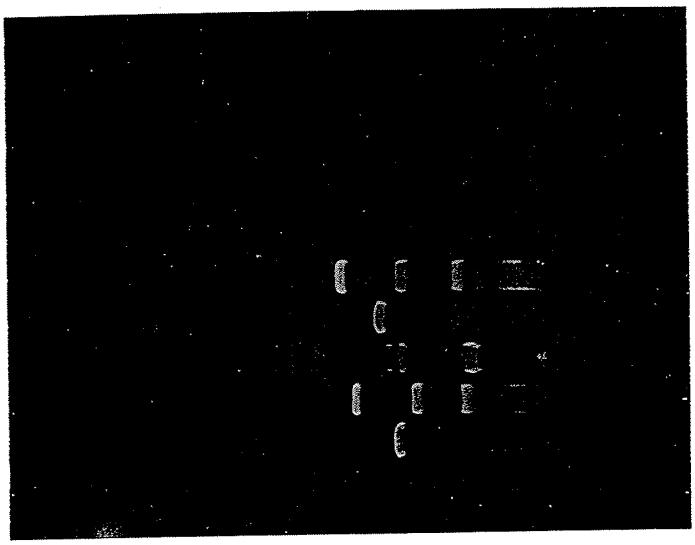
15 × 20 cm gel (Bio-Rad) in TBE, 150 for 3 or 4 hr / 100

1% Agarose
1.5% SeaPlaque

run until front dye is out

start 12:20 at 104 V 50 mA
12:45 106 V 56 mA
~
18:00 staining (for 30 min)
18:40 destaining

19:30



uncut COMP

BstXI cut COMP

λ marker

uncut PXM

BI cut PXM

Vector preparation

pXM ~~CDM8~~ cut ~ EcoRI

plasmid	20 ul (20 ug)
REnt 3	10 ul
EcoRI	5 ul (50 units)
water	65 ul
	100 ul

10:45 ~

CDM 8 cut ~ BstXI

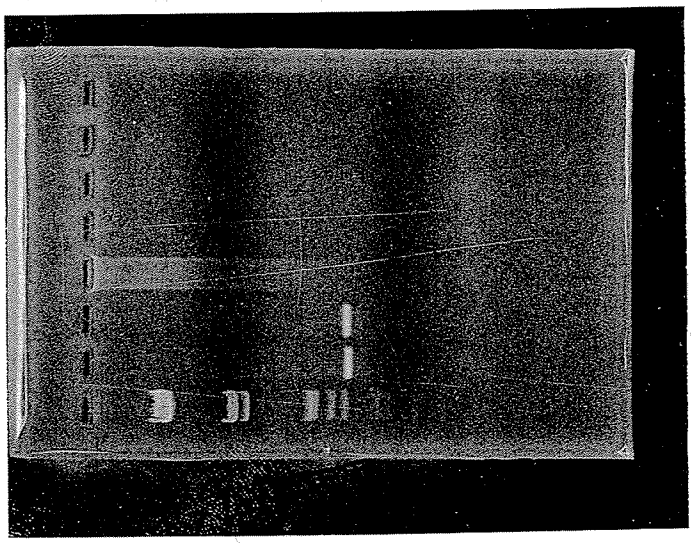
plasmid	20 ul
NEB buffer 3	10 ul
water	65 ul
BstXI	5 ul
	100 ul

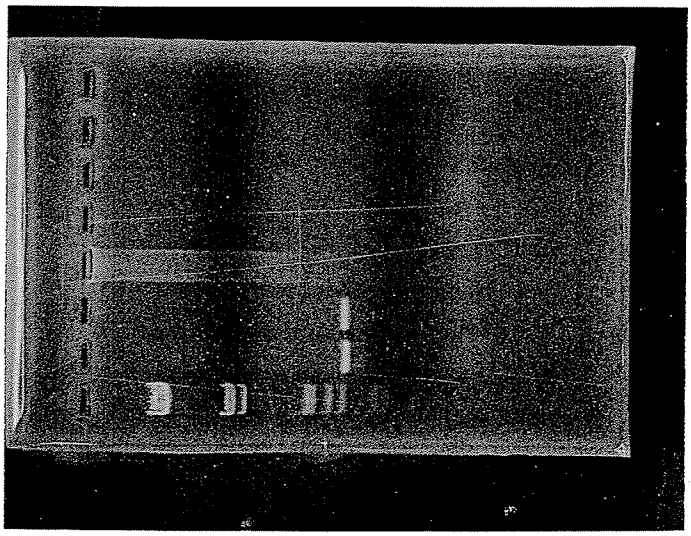
11:28 ~ at 55°C ~

12:20

CIP treat $\frac{+}{-}$

- 68°C 45 min in the presence of 10mM EGTA
- hot phenol 60°C extraction 5 min twice
- chloroform extraction at R.T.
- EtOH prep.





1. Negative control
2. Silver - New 180 ml
3. " " 30 ml
4. " old 30 ml
5. heterozygote
6. C57BL
7. C3H
8. λ marker 5 ml (250 ng)

(if concentration is 1 μg/μl) then $\frac{1}{\#b \times \frac{649}{2}} \times 10^6 = \mu M = \text{pmole}/\mu l$ (3081.7 / #b)

9

PCR

Y0 2028 buffer 10X

Y.2016 MgCl2 50mM

Silver-old

Silver-new

C57BL

(Silver + C57BL) F1

C3H

* 30 μl reaction each × (5 reaction + 1 negative)

= 180 μl (- 6 = 174 μl)

10X buffer	18.0 μl	
MgCl2 (50mM)	5.4 μl	(1.5 mM final)
dNTP (2mM)	18.0 μl	(0.2 mM final)
primer (S1283)	1.0 μl	(0.71 pmole/μl final)
" (S1284)	1.0 μl	(0.69 pmole/μl final)
	43.4 μl	
water	129.6	
Taq polymerase	1.0 μl	(5 units)
	174.0	

divide 29 μl × 6

1. Blank 2. Silver-new 3. Silver-old 4. B57BL 5 F1 6 C3H

genomic DNA 1 μl

[redacted]

50 μg/ml final conc

Dr. Park's # 8, 16, 26 + two more : 5 samples

silver : 50 μl + 350 μl of TE/SDS/proteinase K buffer

→ 65°C > 1 hr. → Chloroform extraction 3 times → 2 Vol. EtOH (layering) → spooling

2 Samples

hetero :

C57BL 1

C3H 1

8 Samples

+ 1 negative control

10 Samples

- proteinase K digestion 17:05 ~ 18:05 ~ 20:25

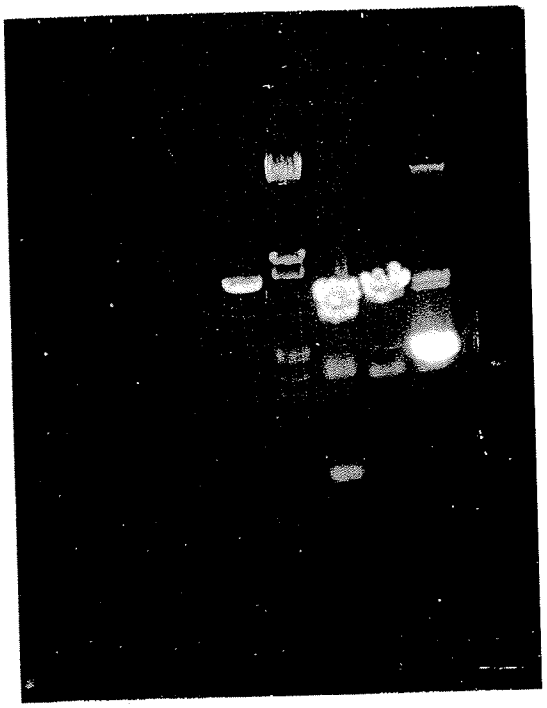
⑤ uncut Jurkat 500

④ Jurkat 500 cut ∑ RI

③ Jurkat 500 cut ∑ RI & HIII

② λ marker 250 ng (5 ml)

① CDM8/BstXI cut, purified on 5-20% KOAc (1 ml out of 200 ml)



300 ng/ml x 200

.6 mg

60 ml

[redacted]

Test cut pGEM 7Zf + Jurkat 500 (in Sma I site) (inactivated)

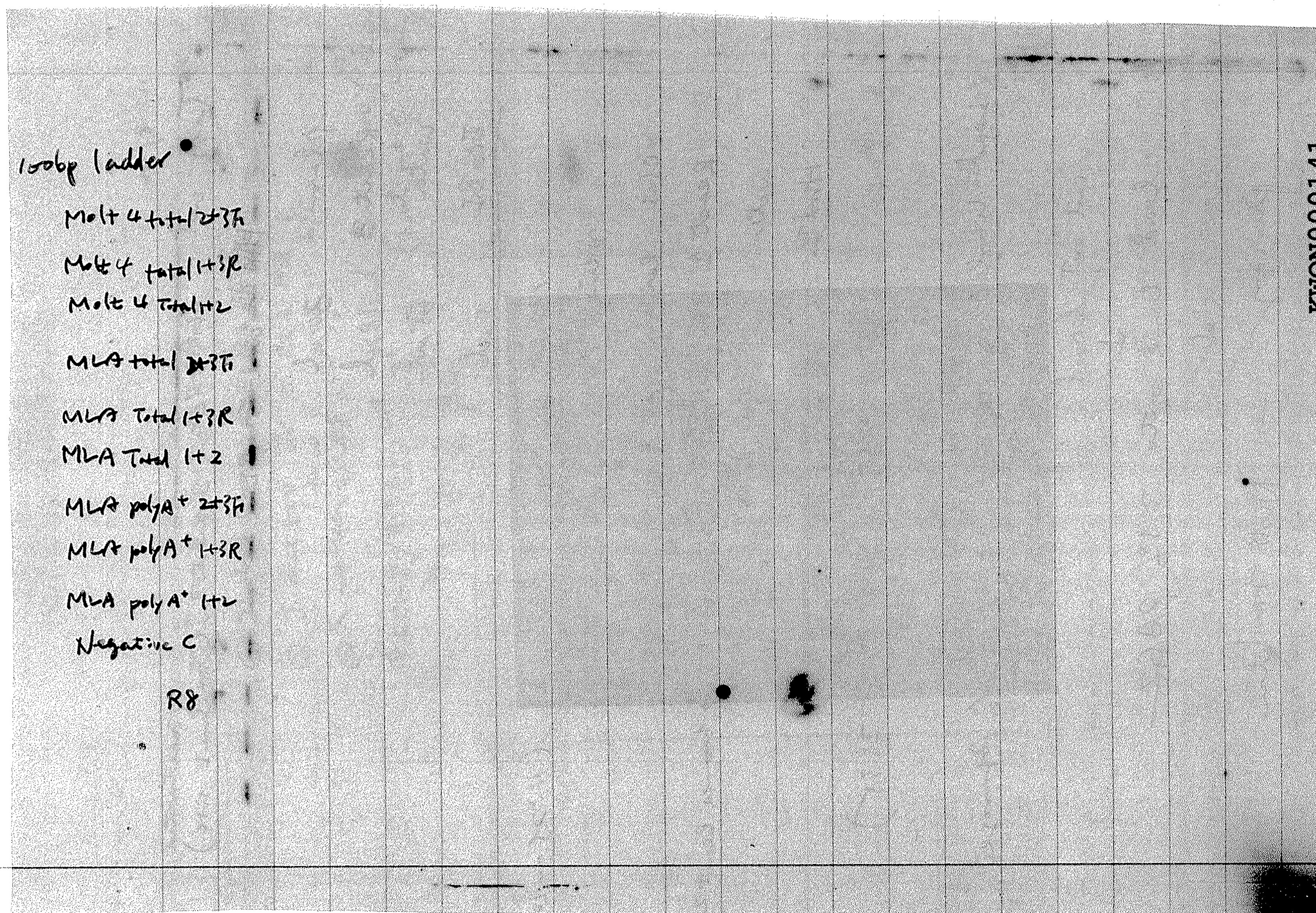
& HindIII and EcoRI

plasmid	30 µl (40 ng)
React 3	10 µl
water	55 µl
EcoRI	5 µl
	100 µl at 37°C 1 hr (11:25 ~ 12:43)

verify cut on Agarose GE.

Cla I Reaction	100 µl
React 1	10 µl (with React 3 → becomes React 2)
water	85 µl
HindIII	5 µl
	200 µl (12:55 ~ 2:35)

- Load whole Rx mixture onto 1% Agarose
  ↓
  cut out band
  ↓
  load band onto 3.5% PAGE
  ↓
  purify → Nick translation


100bp ladder
Molt 4 total 2+3R
Molt 4 total 1+3R
Molt 4 Total 1+2
MLA total 2+3R
MLA Total 1+3R
MLA Total 1+2
MLA polyA+ 2+3R
MLA polyA+ 1+3R
MLA polyA+ 1+2
Negative C
R8

[redacted]

labelling of 4-1BB(1.2kb) by Nick-Translation

4-1BB (1.2kb)	1 ul (100 ng)	1	1
NT buffer	5 ul	5	5
0.1 M DTT	2 ul	2	2
dGTP (10mM)	1 ul	1	1
dTTP (10mM)	1 ul	1 dCTP 1	1
[^{32}P] dATP	10 ul	10	10
[^{32}P] dCTP	10 ul	—	20
DNAase/pol	2 ul	2	2
water	~~18~~ ul	27	4 : 12 ~ 8
	50 ul	at 16°C	1.5~2hr

12 : x2 ~ 14 : 20

$$\frac{3\times10^{6}\ \text{cpm/ul} \times 100\ \text{ul}}{\text{~~100ng/100ng~~}} \times \frac{1000\ \text{ng}}{100\ \text{ng}\cdot\mu\text{g}} = 3\times10^{8}\ \text{cpm}/\mu\text{g}$$

hybridization 15×20 cm NYTRAN

5M NaCl 10 ml

10% SDS 5 ml

150 mg/ml S.S. DNA (10 mg/ml × 750 µl) 750 µl
× 50 ml = 7.5 mg

Probe 3×10^6 cpm/µl [redacted] 50 ml × 10^6 cpm/ml

$= 5 \times 10^7$ cpm

5×10^7 cpm / 3×10^6 cpm/µl ≃ 20 µl

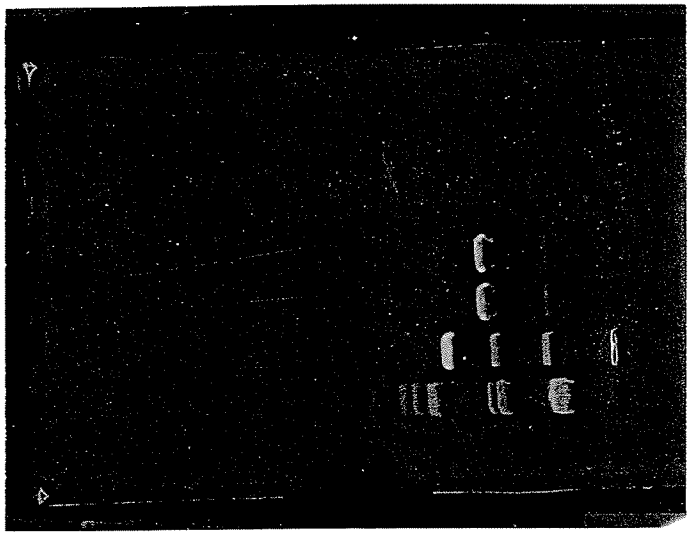
at 65°C O/N

wash 1. 2×SSC + 1% SDS at R.T.
(Total 500 ml)

2. 2×SSC + 1% SDS at 42°C
for 15 min.

expose film at -70°C

develop after 18 hrs



Agarose 1%

BstXI cut (300ng)
EcoRI cut (")
uncut pCDNA1
λ 450ng

[redacted]

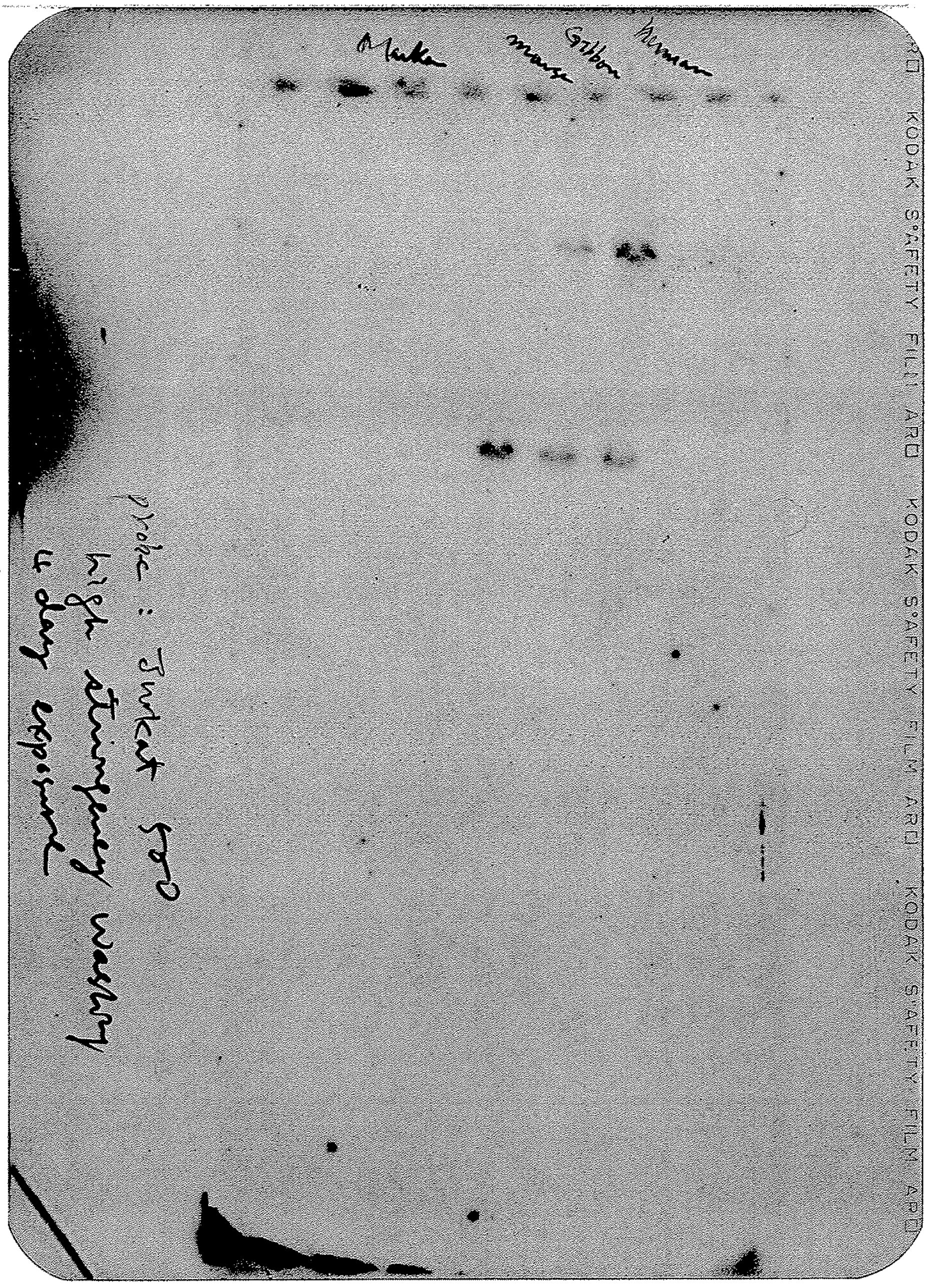
PCDNA test cut

· dilute DNA (4 ng/μl) 1 μl in TE 19 μl (1:20 dilution)

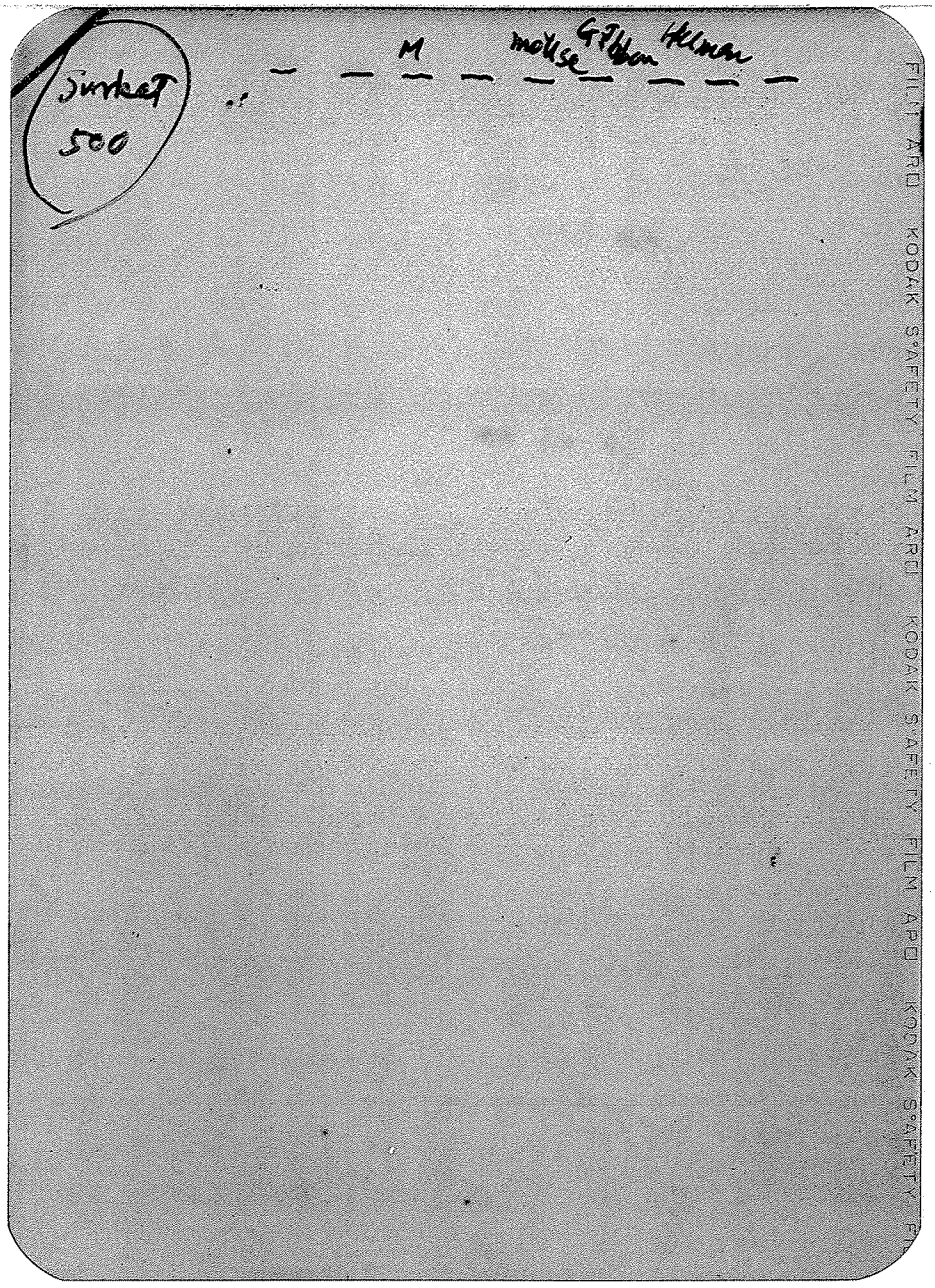
Rx 1	diluted DNA (~~2~~00 ng/μl)	3 μl (600 ng)	
	NEB buffer	2 μl	
	water	14 μl	
	BstXI	1 μl	
		20 μl	50°C · 17:55 ~20:00
Rx 2	diluted DNA	3 μl (600 ng)	
	React 3	2 μl	
	water	14 μl	
	EcoRI	1 μl	
		20 μl	37°C 17:50 ~20:00

[redacted]

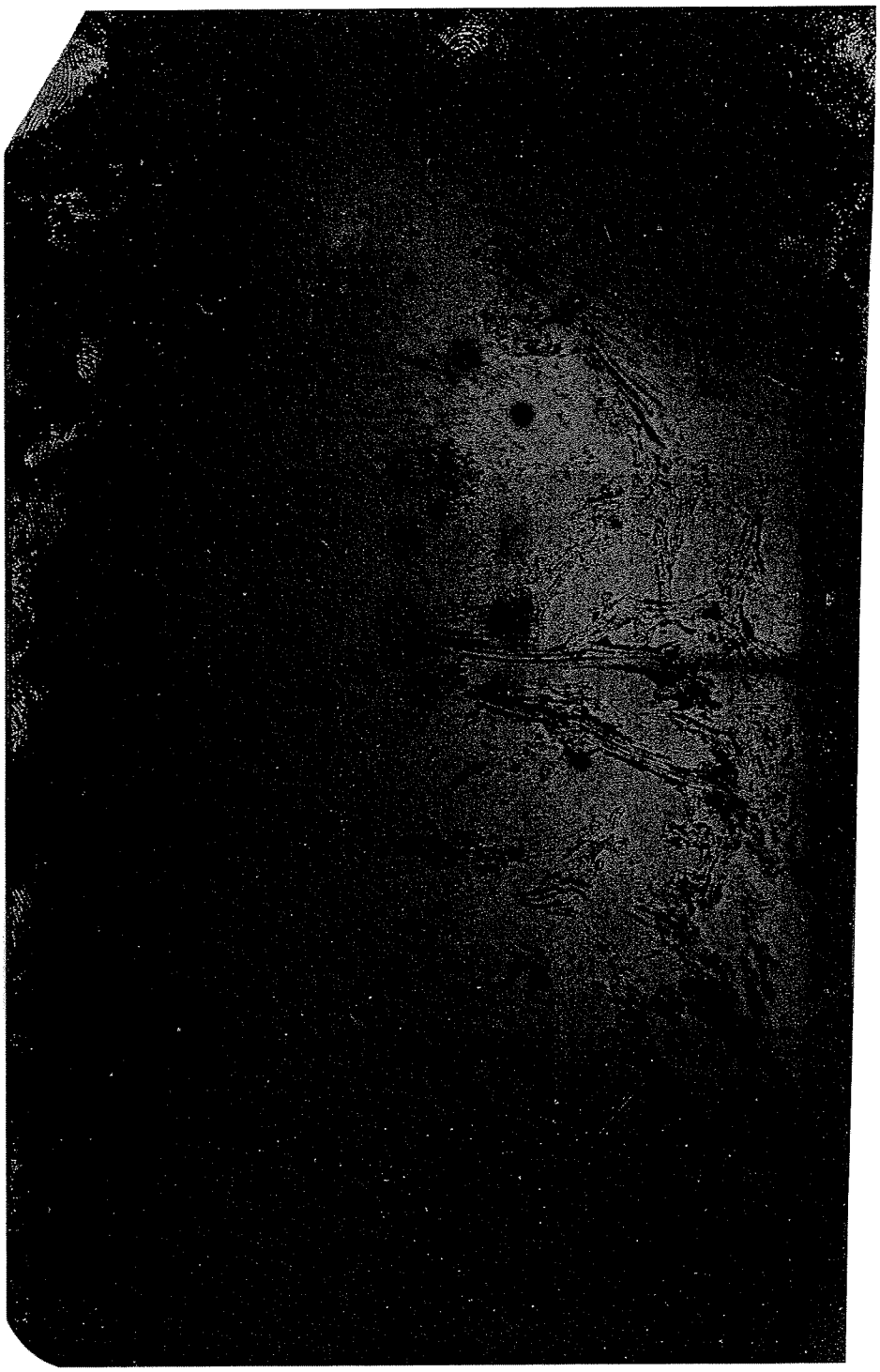
Membrane strip [0.2% SDS
10 mM Tris pH 8.0 (50 mM by fault) 85°C 2h 20:40 ~ 22:40


mouse
Gibbon
human
probe : Jurkat 500
high stringency washing
4 day exposure
KODAK SAFETY FILM AR

KWON000146



KWON000147



[redacted]

Nick translation of Junkat ~~500~~ PCR fragment (PAGE purified)

DNA 1 μl (100 ng)

follows [redacted] 4-IBB labelling protocol (page 15)

at 16°C 16:25 ~ 18:25

4.7×10^6 cpm/μl × 30 μl ≅ 1.2×10^8 cpm / total

$\frac{1.1 \times 10^6 \text{ cpm/μl} \times 25 \text{ μl}}{4.7 \times 10^6 \text{ cpm/μl}} = 5 \text{ μl}$

Sp. act. 1.2×10^8 cpm/μg

8 × 17.5 cm membrane : 140 cm² → 28 ml

$50 \text{ ml} \times \frac{6\times}{20\times} = 15 \text{ ml}$ (of 20× SSC)

$50 \text{ ml} \times \frac{0.5\%}{10\%} = 2.5 \text{ ml}$ (of 10% SDS)

$\frac{100 \text{ μg/ml}}{10 \text{ mg/ml}} \times 50 \text{ ml} = 500 \text{ μl}$ (of 10 mg/ml SS-DNA)

cycle profile

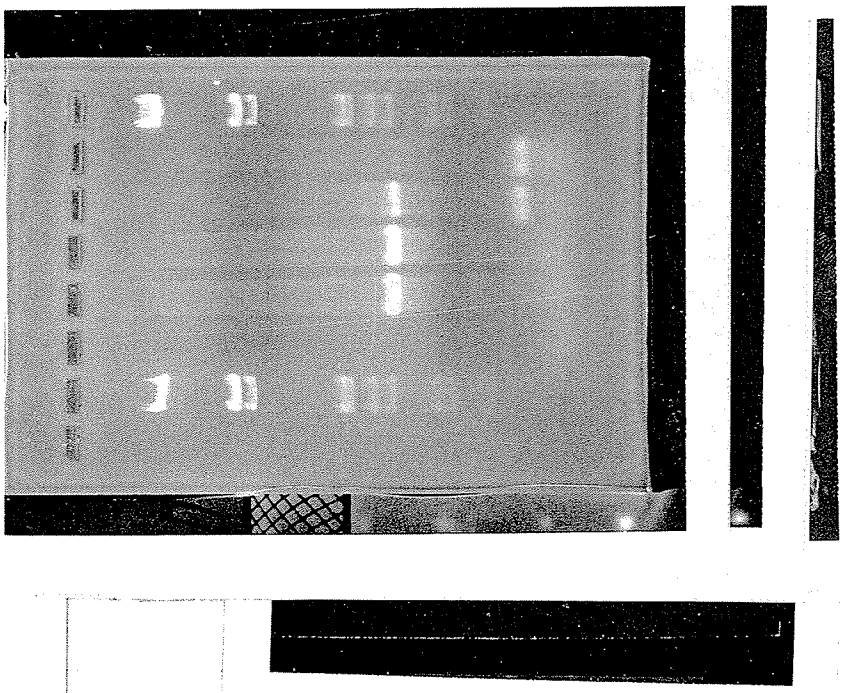
user 14. 94°C 2min

15. 94°C 1min 55°C 1min 72 1min

16. 94°C " " " " 2min

17 72°C 10min

7 4°C



PCR

template ① silver
(9) ② heter
⑤ ⑥ ⑦ ⑧ ⑨
silver (Dr. Park's # 1, 8, 11, 26, 38)
③ C57BL
④ C3H

30 ul/reaction × (9 reactions + 1 negative control)
= 300 ul (– 1ul of template ×10 template = 290 ul)

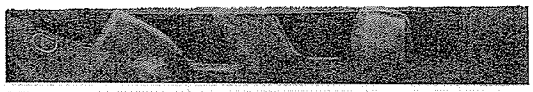
Master mix

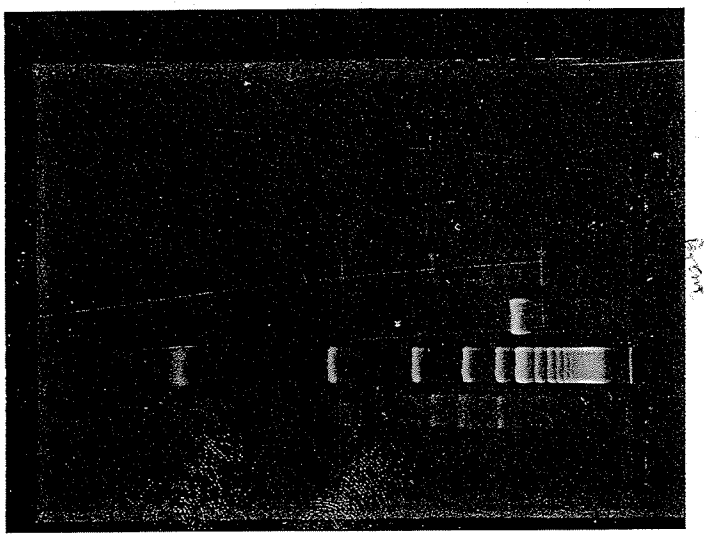
10X buffer	30.0 ul	
MgCl2 (50mM)	9.0 ul	(1.5mM final)
dNTP (10mM / 5mM)	~~12.0~~ 6.0 ul	(0.2mM ")
primer (S1283)	2.0 ul	(~0.9 pmole/ul)
" (S1284)	2.0 ul	(")
Subtotal	~~55.0~~ 49 ul	
Taq	2.0 ul	(10 units)
water	~~235~~ 239.0 ul	
	290.0 ul	

- divide 29 ul into 10 tubes that contain 1ul template on the wall
- add paraffin oil (3 drops)
- vortex → spin → cycle

MIP Brent

Brent steel





Brent 900

steel (M) 1100 ✓

(N) 480 ✓

(J) 380

MIP (m) 900

(l) 750

(K) 700

(I) 550 ✓

330

310

220

~~200~~

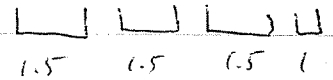
150

@ [redacted]

PAGE purification of Steel, Brent (pmel17), and MIP PCR

EOH ppt of 100 ul of PCR Rx. → redissolve in 20 ul water

(add Glycogen or linear PA)



[redacted]

polishing the end (as in [redacted])

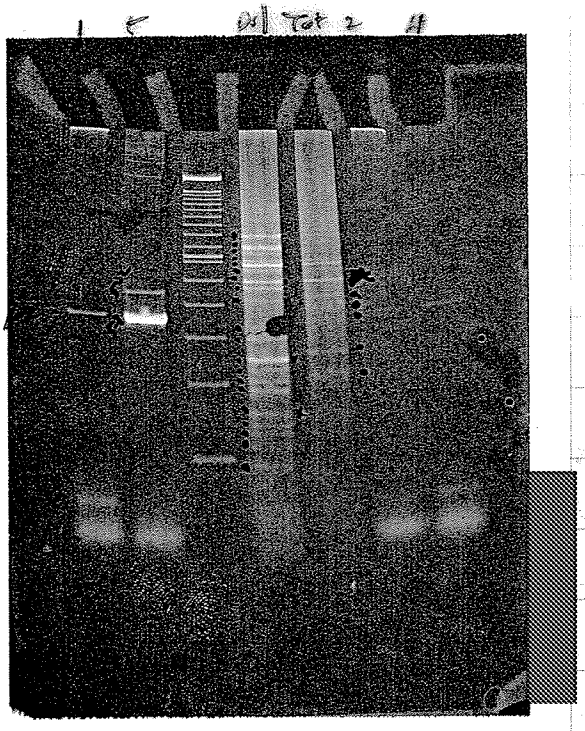
DNA	20 ul (in b.b.w.)	
10X buffer	10 ul	master mix
water	68 ul	80 ul x 13 = 1040 ul
Kinase	1 ul	
Klenow	1 ul	
	100 ul (x 13 = 1300 ul)	

10X buffer	130 ul
water	890
Kinase	10 ul
Klenow	10 ul
	1040 ul

(6)

8:45 ~ 9:45

mouse pMEL17 cDNA



1. <100, 1-2L

[redacted] PCR

template

① silver ② hetero ③ C57BL ④ silver cDNA ⑤ mouse pMZ-L17cDNA

100 µl/reaction × (5 reactions + 1 negative control (half vol.))
= 550 µl (= 1 µl × 5.0 ≠ 545.5)

master mix

10× buffer	50 µl
MgCl₂ (50mM)	20 µl (2mM final)
dNTP (10mM)	10 µl (0.2mM ″)
primer (S1283)	4 µl (≈0.4 pmole/µl)
primer (S1284)	4 µl (″)
subtotal	88 µl
Taq	3 µl (5 units)
water	404
	495
	545.5

divide 99 µl each (×5)

5:03 ~ 5:33 ~ 6:18

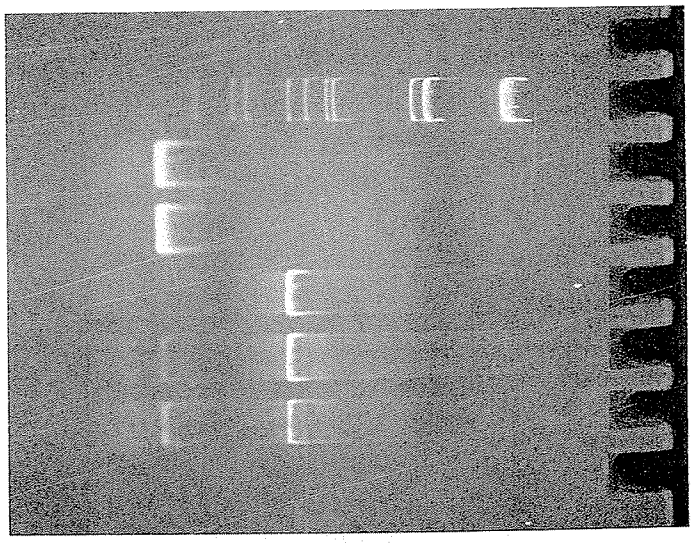
[redacted]

Preparations for cDNA synthesis

1. PXM/RI CIP treatment

~ 20 mg PXM/RI (page 5) p/p extracted by EOH ppt
dissolved in 90 µl of Tris (pH 8.4) according to Maniatis (pH 8.3)
aliquot 1 µl and save
add 10 µl 10X CIP buffer (10 mM ZnCl2
10 mM MgCl2
100 mM Tris (pH 8.4)
add 1 µl (1 unit/µl) of BM CIP
incubate at 37°C for 30 min.
(add 2 µl of 0.5M EGTA (final 10mM)
and incubate at 68°C for 45 min (or 65°C (2) for 1hr)
(add pre-heated (55°C) phenol/chloroform,
vortex and incubate at 55°C for 5 min.
spin and transfer upper aq. layer to a new tube
repeat
EOH ppt

5 4 3 2 1



[redacted]

PCR repeat (page 25)

template ① silver ② hetero ③ C57BL ④ silver DNA ⑤ mouse pMEL17 cDNA

Reaction volume [redacted] same as page 25

Cycle profile

1	cycle	user 14	94°C	2 min			
4	cycle	user 15	94°C	1 min	50°C	1.5 min	72°C 2 min
11	cycle	user 16	94°C	1 min	55°C	1.0 min	72°C 1 min
15	cycle	user 17	94°C	1 min	55°C	1.0 min	72°C 2 min
1	cycle	user 5	72°C	10 min			
1	cycle	user 7	25°C	R.T.			

50 ul/reaction × 5 reactions

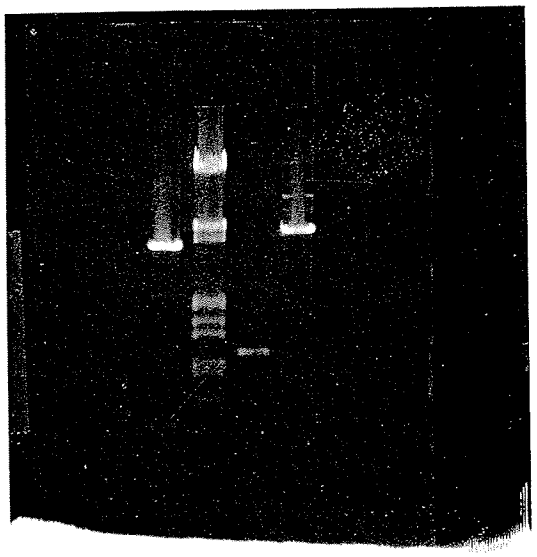
= 250 ul (- 1 ul/template × ~~5~~ template = 245 ul)

master mix			
	10X buffer	25 ul	
	$MgCl_2$ (50mM)	7.5 ul	(1.5 mM final)
	dNTP (10mM)	5 ul	(0.2mM ea)
	primer (S1283)	2.0 ul	(1 pmole/ul)
	" (S1284)	2.0 ul	(")
	subtotal	41.5 ul	
	Taq	2.0 ul	
	water	201.5 ul	
		245.0 ul	

(divide 49 ul × 5 tube
add 1 ul of template
add paraffin oil

SAMPLE	A320	A280	AD
1.0000	0.0000	0.0000	0.0
2.0000	0.0043	0.0722	0.1
3.0000	-0.001	-0.001	-0.
4.0000	-0.001	0.0477	0.0
5.0000	-0.001	0.0480	0.0
6.0000	0.0046	0.0074	0.0
7.0000	0.0040	0.0030	0.0
8.0000	0.0077	0.1348	0.2
9.0000	0.0075	0.1344	0.2

CDM 8 4-1BB/RI pXM



CDM 8 : Stuffer remains

4-1BB/RI

pXM : Some insert remains

Test ligation of { CIP Tx PXM/RI vector
CDM8/BstXI

1. PXM/RI (111 ng/ul)	1 ul	1 ul
4-1BB (15.7 ng/ul)	1.7 ul	—
5X BRL buffer	4.0 ul	4.0 ul
T4 DNA Ligase	1.0 ul	1.0 ul
water	12.3	14.0 ul
	20.0 ul	20.0 ul

Vectors are not prepared well!!

⇓

repurified → p39

[redacted]

Dot blot of MLA [Total RNA / poly A] PCR products

→

4-1BB	PXM	~~pCDNA1~~	pCDM8	Ladder	λ	poly → 98	110	120	135	150	190
210	220	240	295	300	410	490	490	550	570	600	650
700	poly ← → Tot 780	210	270	350	380	410					4-1BB

3ul each of pcr product (out of 20 ul) dotted

- 4-1BB 50 ng
- PXM 100 ng
- pCDM8 200 ng
- Ladder 300 ng (0.3ul)
- λ 150 ng

1. after application float on D.D.W
2. denature for 5'
3. neutralization for 5'
4. rinse in 2XSSC
5. partially dried → Stratalinker

SAMPLE	A320	A280	A260	280/260	260/280	PROTEIN	NUCLEIC ACID	
1.0000	0.0000	0.0000	0.0000	******	******	0.0000	0.0000	DNA: D.W
2.0000	0.0188	0.0399	0.0662	0.4464	2.2401	pMEL17/PvuII 6:57	2.2197	22.197 mg/ml
3.0000	0.0000	0.0000	0.0000	******	******	0.0000	0.0000	
4.0000	0.0050	0.1120	0.2163	0.5062	1.9753	pCDM8 6:66	3.4350	97.536 mg/ml
5.0000	0.0021	0.0025	0.0026	0.0462	1.1518	1.2091	0.0169	
6.0000	0.0023	0.0026	0.0036	-0.182	-5.500	-0.935	0.0611	
7.0000	0.0013	0.0000	0.0000	1.0000	1.0000	-1.017	-0.034	
0000	0.0051	0.0432	0.0812	0.5005	1.9979	pcDNA1.4548 6:57	3.4146	34.146 "
00	0.0000	0.0000	0.0000	******	******	0.0000	0.0000	
0	0.0161	0.1060	0.1913	0.5129	1.9501	pXM 6.7527 6:57	7.7858	77.858 "

[redacted]

ligation of BstXI cut pCDM8 & pCDNA1
with adapted pVUII fragment of pMEX17 (or pXM frg)

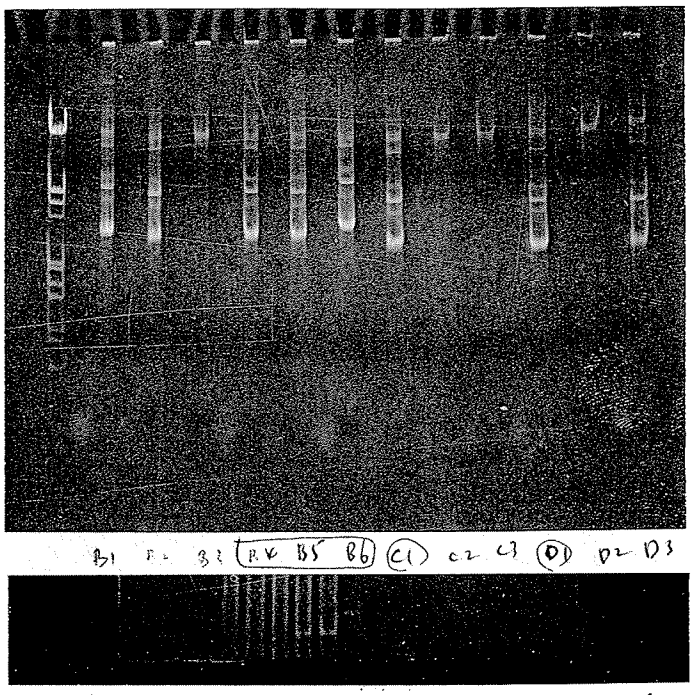
1. Adaptor ligation

pVUII fragment (22 ng/μl)	2 μl
BstXI adapter (0.5 ng/μl)	1 μl
5X BRL lig. buffer	4 μl
water	12 μl
T4 ligase	1 μl
	20 μl at 16° 1 hr

11:45 a 01:00

- at 65°C 10 min
- add NaI (gene clean kit) 150 μl
- add 2 μl of glassmilk (01:17)
- follow gene clean procedure
- elute twice → total 20 μl

CDNA



Transfo
In Vitro
add
divi
on
add

0.3ml
(0.3ml)
μl
tube
cleaned)

on

heat shock at 42°c water bath for 65 seconds
on ice for > 2min.
add 300 250 μl of SOC medium (provided by Invitrogen)
37°c on the wheel for 1 hr.
plate whole thing on Amp-LB plate
(g.h; before plating add 3ml LB and plate 100 μl each)

100ng vector

25ng

40

104000

1x10⁵/ng

a: nothing ~~PCOM8~~ 261
b: ~260 pcDNA1
c: 335
d: 279
f: 205
g: ~560 × 33.5 = 18,200/ng
h: nothing

amount supplied

a: eg cDNA both in pCDNA3

10⁷/ng

KWON000164

[redacted]

ligation of adapter-pmel17/pvuII ⊂ [CDM8 / pCDNA1]

1. gene-cleaned adapter-pmel17/pvuII ~ 10 ul (~20 ng)

ⓐ CDM8 (97 ng/ul) ⓑ pCDNA1 (34 ng/ul)	1 ul	3 ul
5X lg. buffer (BRL)	4 ul	4 ul
water	vector alone 4 ul + self-ligate (14)	2 ul
ligase (T4 DNA ligase, BRL) ⓔ	1 ul	1 ul (ⓕ (12))
	20 ul	20 ul

at 16°C

* control : pmel17/pvuII in place of adapter-pmel17/pvuII

pmel17/pvuII (22 ng/ul)	1 ul	1 ul
ⓒ CDM8 (97 ng/ul) ⓓ pCDNA1	1 ul	3 ul
5X lg. buffer	4 ul	4 ul
water	13 ul	11 ul
ligase	1 ul	1 ul
	20 ul	20 ul

at 16°C

2. Transform [CDM8 / pCDNA1] X
- vector alone
- vector + frag.
- vector + adapter + frag
- uncut vector (1 ng)

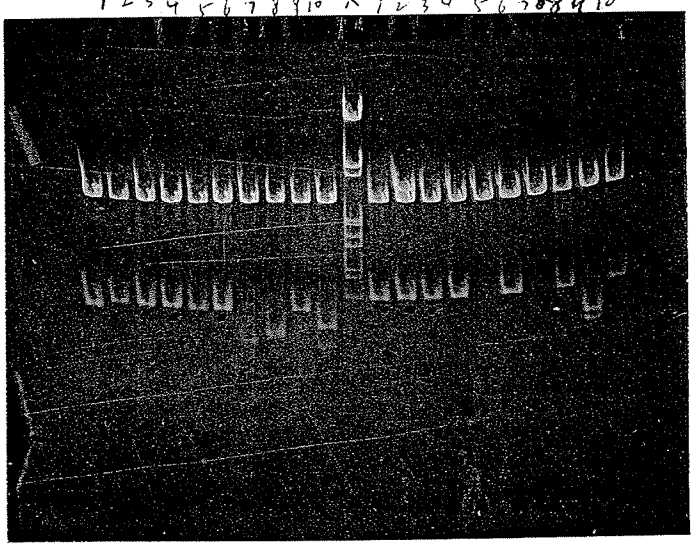
(8 ng)

ⓖ CDM8 ⓗ pCDNA1

ligation of pXM/RI · CIP

	(150 ng)		(120 ng)
1. pXM/RI (78 ng/μl) CIP	2 μl	2 μl	1 μl
4-1BB (16 ng/μl)	2.5 μl	—	—
5X BRL lig. buffer	4 μl	4	4
water	10.5	13 μl	14
T4 ligase (BRL)	1 μl	1	1
	20.0 μl	20 μl	20 μl

✱ pXM/RI CIP - not Tx 1 μl



: 1, 2, 3, 4, 6, 8, 10	: 1
: 9	: 2, 3, 4, 5, 6, 9
: 5, 7 (no insert)	: 7
	: 8
	: 10

[redacted]

digestion of MIP400 & MIP500 clones (10 each X 2)

- mastermix I for 20 µl x 20 = 400 (- 5 µl of miniprep x 20 = 300)

React 3	40 µl
water	240 µl
EcoRI	20 µl
	300 µl

- divide into 20 used & washed tubes
- add 5 µl of minipreps
- mix and at 37°c for 2hr
- take 10 µl separate

Into remaining 10 µl add 10 µl of mastermix 2

master mix 2

React 1	20 µl
water	170
HindIII	10 µl
	200 µl

mix and incubate for 1 hr at 37°c

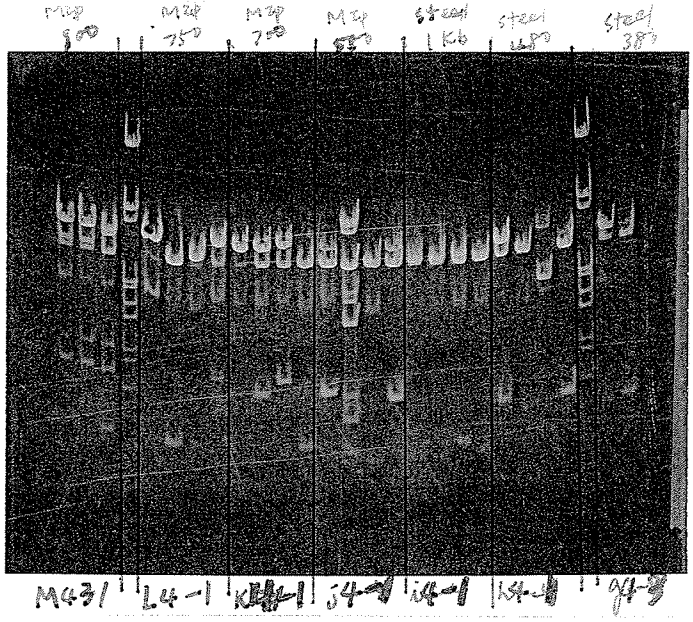
take 10 µl and run gel

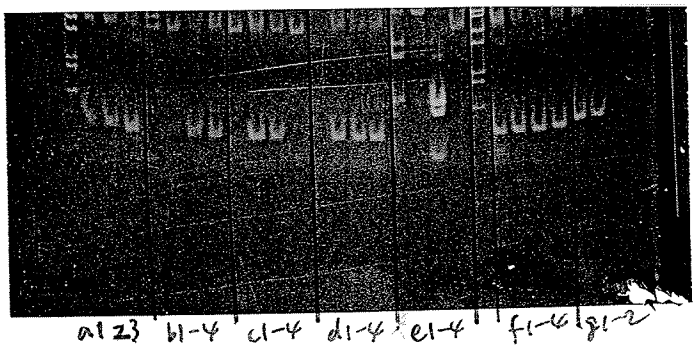
* Transform XL-1 blue ⓒ ligation mixture of polished PCR products of page 77 & 47

page 77 (steel 1Kb, 480, 350 (i, h, g)
MIP 900, 750, 700, 550 (m, l, k, j)

page 47 (⇒)

* pick 4 colonies ~~each~~ from each plate
- prepare plasmid
digest with





[redacted]

ligation of pcr products
Steel 1Kb, 480, 380 (7 fragment)
[MIP 650, 700, 750, 900

ligation

~~7X~~ 20 μl = 140 μl (- ~~[redacted]~~ 10 μl × 7 = 70 μl)

5x buffer	28 μl	
vector	1 μl	(pEGFP-N3/sma2 CTP Tx)
water	36 μl	
T4 ligase	5 μl	divide into 7 tubes (10 μl each) add pcr frag. (10 μl each)
	70 μl	at 20°C 65°C inv

[redacted]

ligation of pcr products from [redacted] and [redacted]

[redacted] (page 29) Silver genomic 1-2 (350bp)
mouse pMEL17 cDNA 350bp 450bp 5-1, 5-2

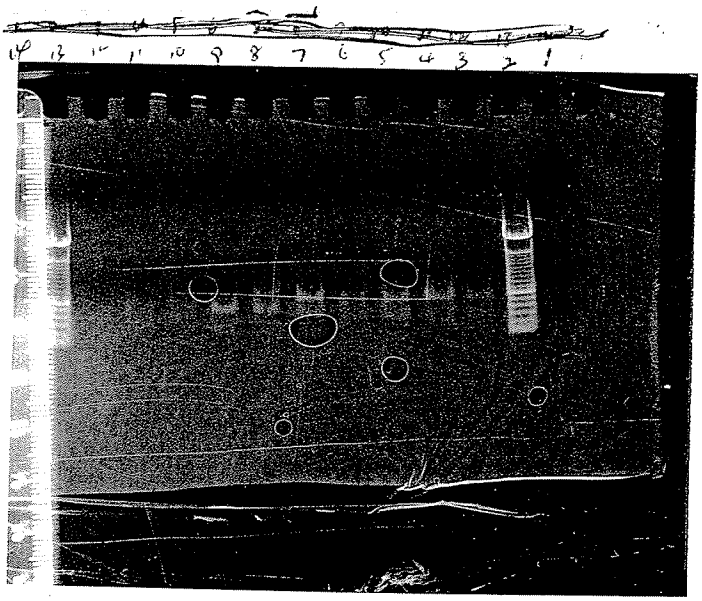
[redacted] (page 33) silver cDNA 4 (350 bp)
silver genomic 1 (1.2 kbp and 350bp)

6 X 20 μl = 120 μl (- 10 μl X 6 = 60 μl)

a 1-2 half (10 μl)
b 5-1 2 μl
c 5-2 half (10 μl)
d 4 2 μl
e 1 (1.2Kb) half
f 1 (350) half

5X buffer	24 μl
vector	1 μl
water	32 μl
T4 ligase	3 μl
	60 μl

divide into 6 tubes 10 μl each
add ⊗ repaired frag.
at 20°C



prehybridization

6X SSC

5X Denhardt

1% SDS

150 ug/ml SSDNA

at 7:20 at 65°C

8:20

hybridization

6X SSC

5X Denhardt

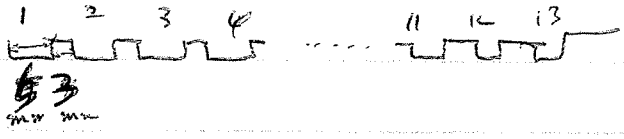
1% SDS

150 ug/ml SS. DNA

4-1BB probe 5×10^6 cpm/ml

at 37°C

[redacted]



total
101 mm

1. ~~ladder~~ 4-1BB
2. ~~4-1BB~~ ladder
3. 98 220 550
4. 110 240 570
5. 120 295 600
6. 135 320 650
7. 150 410 700
8. (190) 470 780
9. (210) 490
10. 220 380
11. (270) 410
12. 350
13. ~~4-1BB~~ ladder
14. 4-1BB

poly A+ mix 7 µl of each frag

total RNA

KWON000172

SAMPLE	A320	A280	A260	280/260	260/280	PROTEIN	NUCLEIC ACID	
1.0000	-0.001	0.0000	0.0010	0.5098	1.9615	0.0692	0.0909	
2.0000	0.0049	0.0424	0.0801	0.4989	2.0043	1.2821	3.3794	pRC/CMV (BstXI) = water 2.5 : 57.5 ⇒ 84 ng/μl
3.0000	-0.001	-0.001	0.0000	-0.080	-12.50	-0.881	0.0658	
4.0000	0.0293	0.0520	0.0678	0.5894	1.6966	6.0585	1.6039	
5.0000	-0.001	0.0000	0.0000	1.0000	1.0000	0.9536	0.0323	
6.0000	0.0119	0.0201	0.0300	0.4523	2.2108	-0.997	0.8410	
7.0000	0.0112	0.0205	0.0295	0.5098	1.9614	0.6212	0.9143	
8.0000	-0.002	-0.002	-0.002	-2.000	-0.500	-0.618	0.0216	
9.0000	0.0174	0.0338	0.0498	0.5222	1.9148	1.6751	1.3883	] pMEL 17-1/PVUII BstXI : water 5 : 55 ⇒ 16.8 ng/μl
10.000	0.0181	0.0340	0.0495	0.5077	1.9699	0.9589	1.3994	

[redacted]

cut pRC/CMV ~~c~~ BstXI

plasmid	15 ul (15 ug)
NEB #3	10 ul
water	70 ul
BstXI	5 ul
	100 ul

at 50°C [redacted] min 90

~~add BstXI~~

ligation

	①	②	③	④	⑤	⑥
pCDM8	1ul	1 ul	—	—	—	—
~~pRC/CMV~~						
~~pcDNA1~~	—	—	[redacted]	[redacted]	—	—
~~pRC/CMV~~						
pcDNA1	—	—	—	—	2.5	2.5
5X lig. buffer	4	4	4	4	4	4
pREP17 pVUII/BstXI	1	—	1	—	1	—
water	13	14	13	14	11.5	12.5 (16.5+1)
ligase	1	1	1	1	1	1
	20	20	20	20	20	20

9 : 15 (7)

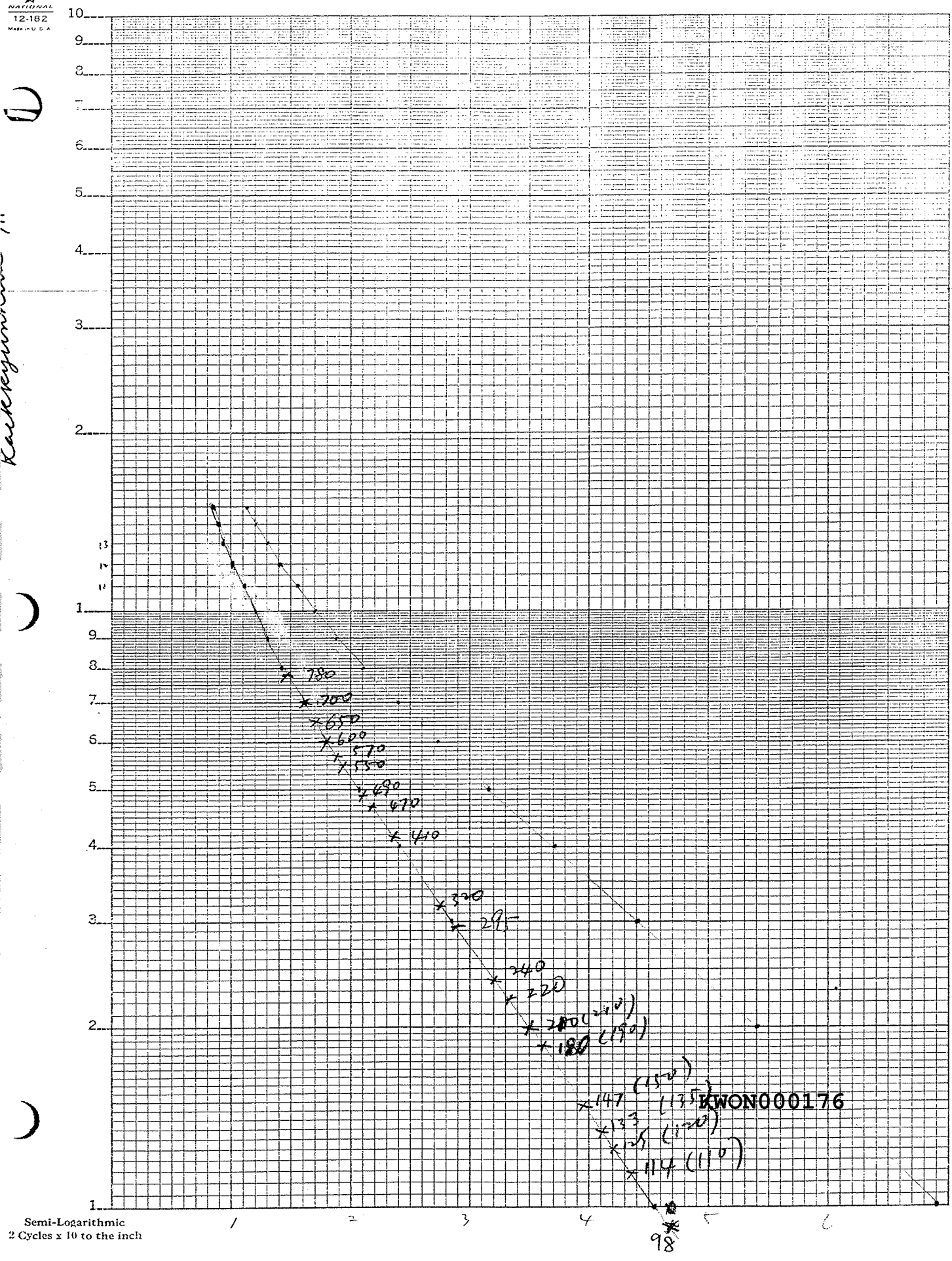
Master mix 20 × 6 = 120 {-(1+4.5) × 6 = 99 ~~108~~}

5x buffer	24
water	~~69~~
ligase	6
	~~108~~ 99 ul (16.5)

Todo

1) stirling 1 kb.
   Dr. Kim has ligate
   plate XL-1 blue

2) all the fragment of mip-pcr
   stirling has been repaired
   & cloned


NATIONAL
12-182
Made in U.S.A.
Karkegunnu 1"
780
700
650
600
570
550
490
470
410
320
295
240
220
180
(190)
147
(135)
133
(110)
114 (110)
98
KWON000176
Semi-Logarithmic
2 Cycles x 10 to the inch


mLApolyA
λ
P
λ